Molecular and Cellular Neuroscience 47 (2011) 274–285

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Regulation of adult neurogenesis by behavior and age in the accessory olfactory bulb

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ARTICLE INFO

Article history:
Received 30 November 2010
Revised 16 April 2011
Accepted 3 May 2011
Available online 10 May 2011

Keywords:
Granule cell
Social odors
Aggression
Aging
Vomeronasal system
Stress
Proliferation

ABSTRACT

The vomeronasal system (VNS) participates in the detection and processing of pheromonal information related to social and sexual behaviors. Within the VNS, two different populations of sensory neurons, with a distinct pattern of distribution, line the epithelium of the vomeronasal organ (VNO) and give rise to segregated sensory projections to the accessory olfactory bulb (AOB). Apical sensory neurons in the VNO project to the anterior AOB (aAOB), while basal neurons project to the posterior AOB (pAOB). In the AOB, the largest population of neurons are inhibitory, the granule and periglomerular cells (GCs and PGs) and remarkably, these neurons are continuously born and functionally integrated in the adult brain, underscoring their role on olfactory function. Here we show that behaviors mediated by the VNS differentially regulate adult neurogenesis across the anterior–posterior axis of the AOB. We used immunohistochemical labeling of newly born cells under different behavioral conditions in mice. Using a resident-intruder aggression paradigm, we found that subordinate mice exhibited increased neurogenesis in the aAOB. In addition, in sexually naive adult females exposed to soiled bedding odorized by adult males, the number of newly born cells was significantly increased in the pAOB; however, neurogenesis was not affected in females exposed to female odors. In addition, we found that at two months of age adult neurogenesis was sexually dimorphic, with male mice exhibiting higher levels of newly born cells than females. Interestingly, adult neurogenesis was greatly reduced with age and this decrease correlated with a decrease in progenitor cells proliferation but not with an increase in cell death in the AOB. These results indicate that the physiological regulation of adult neurogenesis in the AOB by behaviors is both sex and age dependent and suggests an important role of newly born neurons in sex dependent behaviors mediated by the VNS.

Introduction

The ability to find potential mates and properly identify social status among conspecifics is essential for species survival and largely relies on the detection and recognition of social chemosensory cues by the concerted activity of the main olfactory and vomeronasal systems (Baum and Kelliher, 2009). Sensory neurons in the main olfactory epithelium and in the vomeronasal organ (VNO) send their axons to specific regions of the olfactory bulb (OB), the main and the accessory olfactory bulb (MOB and AOB, respectively), where they establish their first synapse onto principal neurons, the mitral and tufted cells (MCs herein). In addition, in the VNO, sensory neurons exhibit differences in pheromone receptor expression and segregated axonal projection to the AOB, giving rise to anatomical and functional subdivisions in the AOB (Halpern et al., 1995; Jia and Halpern, 1996; Sugai et al., 2006). Thus, neurons in the apical layer of the VNO express V1R receptors and project to the anterior AOB (aAOB), while neurons in the basal layer express V2R receptors and project to the posterior AOB (pAOB, Fig. 2A) (Herrada and Dulac, 1997; Rodriguez et al., 1999). This particular arrangement of sensory inputs into the AOB has suggested that these functional subdivisions are involved in the processing of pheromonal information related to species-specific behaviors. For instance, in male mice, presentation of a diestrus female activates neurons in the aAOB (Kumar et al., 1999), whereas male aggressive behavior, exposure of males to females' volatile odors, and the exposure of females to male major urinary proteins induce activation of cells in the pAOB (Brennan et al., 1999; Kumar et al., 1999; Yoshikage et al., 2007).

The most salient physiological mechanism in olfactory processing in the OB is the precise regulation of MCs' activity by inhibitory interneurons. These inhibitory neurons are broadly classified as periglomerular and granule cells (PG and GCs, respectively). A large population of these neurons correspond to GCs, which produce recurrent and lateral inhibition of MCs through dendrodendritic synapses, shaping their output and thereby playing a fundamental role in olfactory processing (Arevian et al., 2008; Schoppa and Urban,

Abbreviations: AOB, accessory olfactory bulb; aAOB, anterior accessory olfactory bulb; aGCL, anterior granule cell layer; BrdU, 5-bromo-2′-deoxyuridine; GC, granule cell; GCL, granule cell layer; HMW, High molecular weight; LMW, Low molecular weight; LOT, lateral olfactory tract; MC, mitral/tufted cell; MOB, main olfactory bulb; OB, olfactory bulb; PBS, phosphate-buffer-saline; pAOB, posterior accessory olfactory bulb; PFA, paraformaldehyde; PG, periglomerular cell; SVZ, subventricular zone; VNO, vomeronasal organ; VNS, vomeronasal system.

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doi:10.1016/j.mcn.2011.05.003

2003). Remarkably, unlike most neurons in the adult mammalian brain, these inhibitory neurons are continuously born throughout life in a process known as adult neurogenesis (Altman and Das, 1965b; Lois and Alvarez-Buylla, 1993, 1994). Neurons born during development and in the adult originate from progenitor cells located in the subventricular zone (SVZ) of the brain, from where they migrate to the OB and become functionally integrated within the OB neuronal network. More importantly, the rate of adult neurogenesis can be greatly regulated by a number of factors including olfactory stimuli and the activity of afferent modulatory systems, suggesting that adult neurogenesis is regulated in the context of olfactory behavioral experience (for a review see Lledo and Lazarini, 2007).

The Vomeronasal system (VNS) plays an important facilitating role in several social behaviors including mating and aggression (Bean, 1982; Clancy et al., 1984; Leypold et al., 2002; Maruniak et al., 1986; Mugford and Nowell, 1970; Norlin et al., 2003; Stowers et al., 2002). Notably, chemosensory information associated with these behaviors recruits the activity of inhibitory neurons in the AOB, suggesting that these behaviors can regulate adult neurogenesis (Sugai et al., 2006; Yoshikage et al., 2007). Here, we use immunohistochemical labeling of newly born cells to show that social behaviors relying on the activation of the VNS regulate adult neurogenesis in the mouse AOB. We found that adult neurogenesis can be differentially modulated across the anterior–posterior axis of the AOB depending of the type of social stimulus. The induction of aggressive behavior in males, via a resident-intruder paradigm, significantly increased neurogenesis in the aAOB of the intruder. In contrast, naive females exposed to male urine exhibited increased adult neurogenesis in the pAOB. Interestingly, we found that the generation of new neurons in the AOB is sexually dimorphic at one month of age, with males having greater numbers of newly born neurons compared to females. However, this sexual difference is not maintained at later ages and adult neurogenesis dramatically decreases with age in both sexes.

Results

Adult neurogenesis in the accessory olfactory bulb in juvenile mice is sexually dimorphic

Anatomical and physiological evidence indicate that in several species the VNS is sexually dimorphic (Dawley and Crowder, 1995; Herrada and Dulac, 1997; Peretto et al., 2001; Segovia et al., 2006; Segovia et al., 1999), suggesting that adult neurogenesis could be differentially regulated in the AOB of male and female mice. To address this possibility we first determined the level of adult neurogenesis in the AOB of sexually mature and juvenile mice. To this end, one-month old mice were injected with BrdU, a marker that is incorporated into the DNA of dividing cells, and labeled neurons in the AOB were quantified 30 days after the BrdU injection. Previous studies have indicated that after 1 month, newly born neurons have already differentiated into mature GCs and PGs within the MOB (Belluzzi et al., 2003; Carleton et al., 2003; Lledo et al., 2006; Petreanu and Alvarez-Buylla, 2002).

In sagittal sections of the mouse brain, the AOB is located in the dorso-posterior region of the OB and can be clearly distinguished from the MOB (Fig. 1A). In the AOB, the mitral/tufted cell layer (MC) and the GC layer (GCL) are separated by fibers of the lateral olfactory tract (LOT), whereas the glomerular layer (GL) faces the ventral prefrontal cortex (Fig. 1A, top right). Mature neurons were recognized by detecting the presence of NeuN, a nuclear marker that is present in post-mitotic and terminally differentiated neurons (Mullen et al., 1992). As shown in Fig. 1B we found abundant NeuN staining in the AOB and MOB, especially in the dense GCL. Confocal imaging analysis revealed abundant BrdU positive (BrdU+) cells throughout the GCL and GL in the AOB and MOB (Fig. 1C). The pattern of nuclear staining with BrdU was variable; some cells exhibited a punctuated pattern while other cells exhibited a more uniform staining pattern (Fig. 1C). Nevertheless, confocal analysis revealed that at all ages the majority of the new cells present in the adult AOB were indeed neurons, since most of the cells that were BrdU+ also stained for NeuN (2, 3 and 7 months; 94 ± 3%; 92 ± 4%, 93 ± 8%, respectively; n = 3 per group, Fig. 1D). Similarly, as previously described most cells in the MOB also exhibited an overlapping staining pattern for BrdU and NeuN at all ages tested (2, 3 and 7 months; 94 ± 2%; 96 ± 1%, 91 ± 2%, respectively; n = 3 per group) as previously reported (Alonso et al., 2006; Mak et al., 2007; Mouret et al., 2009; Rochefort et al., 2002; Veyrac et al., 2009).

Interestingly, at two months of age the level of adult neurogenesis was significantly different between male and females (Fig. 2B). We found that the total number of BrdU+ cells in the AOB was ~40% higher in males than in females (males, 2782 ± 373, n = 5; females 1704 ± 186, BrdU+ cells/mm^3, n = 6; *t*-test, $p<0.03$). Sensory projections from the VNO exhibit a divergent pattern of distribution across the anterior–posterior axis of the AOB, suggesting that these regions may process different chemosensory information (Fig. 2A). Therefore, we determined whether the sexual dimorphism in adult neurogenesis observed at this age was also expressed along the anterior–posterior axis. As shown in Fig. 2B, two-month old male mice exhibited a significantly higher number of BrdU+ cells in the posterior AOB (pAOB) compared to females (males, 2748 ± 406, n = 6; females, 1493 ± 135 BrdU+ cells/mm^3, n = 6; *t*-test, $p<0.02$). The number of newborn neurons found in the anterior AOB (aAOB) was also higher in males compared to females, but this difference did not reach statistical significance within our sample (males, 2817 ± 507; females, 1914 ± 307 BrdU+ cells/mm^3; *t*-test, $p<0.15$). In contrast, we did not observe gender differences in the number of BrdU+ cells in the MOB (males, 6171 ± 406, n = 5; females, 5965 ± 380 BrdU+ cells/mm^3, n = 6; Fig. 2B). The observed gender difference in neurogenesis along the anterior–posterior axis of the AOB could be due to other factors including differences in the number of cells or volume in these two regions. However, as shown in Fig. 2C, the total number of cells was not different between males and females (males, aAOB, $529\times10^3\pm18\times10^3$, pAOB, $517\times10^3\pm32\times10^3$ cells/mm^3; females, aAOB, $614\times10^3\pm47\times10^3$; pAOB, $564\times10^3\pm27\times10^3$ cells/mm^3, n = 3 both). Likewise, the sexual dimorphism in adult neurogenesis is not due to differences in volume between the anterior and posterior subdivisions of the AOB (males, aAOB, $58\times10^6\pm2\times10^6$, pAOB, $52.7\times10^6\pm2\times10^6$; females, aAOB, $57.7\times10^6\pm12\times10^6$; pAOB, $57.7\times10^6\pm11\times10^6$ μm^3; n = 3 both).

Adult neurogenesis in the AOB decreases with age

Olfactory dysfunction is a common pathology reported by the elderly population and is one of the first symptoms manifested by people suffering from neurodegenerative diseases (Kovacs, 2004). The decline in the number of new neurons arriving to the MOB has been suggested as a possible mechanism underlying the decrease in olfactory discrimination related with aging (Enwere et al., 2004), yet a similar age-dependent decrease in adult neurogenesis in the AOB has not been examined. Accordingly, we determined the number of BrdU labeled cells at two, three and seven months of age, in male and female mice, injected one-month earlier. As shown in Fig. 3A, we found a significant decrease in the number of BrdU+ cells at three and seven months compared to the number of cells at 2 months. At 3 months the number of BrdU+ cells in males was decreased by 70%, while at 7 months it was decreased by 90% (2 months, 2782 ± 373, n = 5; 3 months, 825 ± 194, n = 6; 7 months, 258 ± 102 BrdU+ cells/mm^3, n = 5; ANOVA, $F_{(2,13)}=27.9$ $p<0.001$; Tukey HSD 'two vs. three months' and 'two vs. seven month' $p<0.001$). In females, at 3 months the number of BrdU+ cells was decreased by 63%, while at 7 months it was decreased by 99% (2 months, 1704 ± 186, n = 6;